RBM ONLINE

ELSEVIER

Article

Perivitelline threads associated with fragments in human cleavage stage embryos observed through time-lapse microscopy

Ranya Derrick [a,b,1], *Cristina Hickman* [a,b,2,*], *Oriol Oliana* [a], *Thomas Wilkinson* [a], *Danielle Gwinnett* [a], *Lucy Benham Whyte* [c], *Anna Carby* [a], *Stuart Lavery* [a,b]

[a] *Boston Place Clinic, The Fertility Partnership, 20 Boston Place, London, NW1 6ER, UK*
[b] *Imperial College, Hammersmith, London, UK*
[c] *Oxford University, Oxford, UK*

Dr Cristina Hickman is Honorary Lecturer at Imperial College London and Head of Embryology at Boston Place Clinic, The Fertility Partnership, where she designed the first UK clinic to offer embryoscope culture to all patients. She designed six innovative IVF laboratories worldwide and consults on troubleshooting for IVF-related processes.

KEY MESSAGE

Time-lapse microscopy revealed perivitelline threads (PVT) that developed at the two-cell stage and were associated with fragmentation. No correlation was found between PVT and implantation potential or ploidy. Further studies are required to clarify the relationship between PVT and fragmentation, and determine the origin, composition and function of these structures.

A B S T R A C T

Perivitelline threads (PVT) are defined as thin filaments that extend across the perivitelline space connecting the zona pellucida with the oolemma or, in some cases, blastomere membrane. This is the first report of PVT in human embryos. Time-lapse imagery from 525 blastocysts with either tested ploidy, known implantation status, or both, were reviewed for the presence of PVT, the cell stage when PVT were first observed, association with fragmentation, ploidy or implantation potential; PVT were observed in most embryos (404/525 [77%]). The euploidy rate was similar in embryos with PVT (61/152 [40%]) and without PVT (17/35 [49%]). Implantation rates were also similar in embryos with PVT (64/259 [25%]) and without PVT (25/90 [28%]). In the embryos in which PVT were observed, 98% (396/404) developed at the two-cell stage. In most embryos (384/404 [95%]), PVT were observed to directly pull fragments from the embryo. Fragmentation occurred significantly less frequently in embryos without PVT compared with PVT (81/121 [67%] versus 388/404 [96%]; $P < 0.001$). These data suggest an association between PVT and fragmentation. This study is limited in that PVT were not characterized so their nature and origin remain unknown and to be determined in future studies.

* Corresponding author.
E-mail address: cristina.hickman@bostonplaceclinic.co.uk (C Hickman).
[1] First author.
[2] Lead and corresponding author.

http://dx.doi.org/10.1016/j.rbmo.2017.08.026
1472-6483/

Please cite this article in press as: Ranya Derrick, Cristina Hickman, Oriol Oliana, Thomas Wilkinson, Danielle Gwinnett, Lucy Benham Whyte, Anna Carby, Stuart Lavery, Perivitelline threads associated with fragments in human cleavage stage embryos observed through time-lapse microscopy, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.08.026

Introduction

Time-lapse technology captures images every 5–15 min throughout embryo development in culture, allowing for the identification of morphological features that may not be possible to detect through the traditional daily observations of embryos in a microscope outside the incubator. Some of these features have been shown to be associated with embryo viability, such as multinucleation (Desai et al., 2014), reverse cleavage (Desai et al., 2014) and direct cleavage from one blastomere to three or more blastomeres (Zhan et al., 2016).

The EmbryoScope™ culture system to culture human IVF-derived embryos in a clinical practice was used, and perivitelline thread-like structures were observed. Previously unpublished, perivitelline threads (PVT) are projections that extend across the perivitelline space connecting the zona pellucida with the oolemma or blastomere membrane. Anecdotal observations have suggested a possible relationship between PVT and fragmentation.

Fragmentation has been associated with irregular cell division (Prados et al., 2012), initiation of apoptosis in neighbouring cells leading to disruption of blastulation (Sathananthan et al., 1999; Alikani, 2005; Stone et al., 2005; Keltz et al., 2006) and loss of cytoplasmic volume (Alikani et al., 1999; Hardy et al., 2002; Keltz et al., 2006) associated with a reduction in implantation potential (Kovacic et al., 2004). Therefore, fragmentation is an established marker of viability used in most IVF laboratories as part of their embryo selection procedures.

It is proposed that selecting euploid embryos for transfer increases pregnancy rate and decreases abortion rate (Sermon et al., 2016), suggesting that identification of chromosomal euploidy, by using preimplantation genetic screening, can be a useful marker of viability, in addition to implantation potential. Recent studies have demonstrated that morphokinetic features observed during routine clinical culture, are correlated with ploidy (Campbell et al., 2013; Fragouli et al., 2014; Mumusoglu et al., 2017). Time-lapse technology is facilitating the identification and monitoring of morphokinetics, allowing for the assessment of the impact of these features on implantation potential and ploidy.

As PVT have not been reported, their prevalence and clinical significance is unknown.

This preliminary, retrospective observational study aims to determine the following: the incidence of PVT in embryos capable of blastulating; the cell stage at which PVT are first observed; and whether a relationship exists between PVT, implantation, ploidy, fragmentation, or both.

Materials and methods

Ethics

This study was conducted in a clinic with full compliance with the Human Fertilisation and Embryology Authority. This observational study assessed images from embryos from patients who consented to the use of time-lapse technology and for images and data to be included as part of the quality control process to continually improve embryo selection procedure. According to our clinic's research policy ethical approval was not required for this study.

Table 1 – Inclusion and exclusion criteria used to select embryos for analysis.

Inclusion criteria	Exclusion criteria
Blastocysts	Has not had transfer, biopsy, or both
Known implantation data, known ploidy status, or both	Unknown implantation
	If transferred earlier than day 5
	If video is inadequate, e.g. poor lighting, whole embryo not visible
	If unable to make accurate assessment of embryo or perivitelline threads.

Embryo cohorts

This retrospective cohort study took place at a private fertility clinic (Boston Place Clinic, The Fertility Partnership, London). Embryos were selected from a database of 6577 embryos cultured between 2013 and 2015 in the EmbryoScope™ incubator (described in Desai et al., 2014). Only embryos capable of blastulating (n = 525), either with known implantation data (KID) only (n = 338), with KID and known ploidy status (n = 11) or of known ploidy status only (n = 176), were selected for this study (**Table 1**). All embryos meeting the inclusion criteria between 13 September 2013 and 14 November 2013 and between 28 November 2014 and 7 March 2016 were assessed. Videos were analysed from insemination to day 6 and included embryos from IVF and intracytoplasmic sperm injection (ICSI) cycles from patients aged 23–46 years. Type of insemination was assessed as a possible confounding factor using the chi-square test.

A KID-positive embryo is defined as equal or more fetal hearts being observed at 6 weeks via ultrasound scan as the number of embryos transferred, whereas KID negative embryos are defined as transferred embryos not leading to a fetal heart. Ploidy was assessed via Next Generation Sequencing at Reprogenetics UK. Euploid rate is defined as proportion of euploid embryos over total number of embryos with detectable amplification.

To avoid bias, the practitioner assessing embryos for PVT was blinded to ploidy and implantation status. A second operator verified 50 videos for both the presence of PVT and their association with fragmentation. High inter-operator agreement was reached on identification of PVT, association with fragmentation and cell stage (**Table 2**).

Table 2 – Kappa agreement analysis between two observers. Agreement defined as; 'good agreement' = Kappa coefficient 0.6–0.8, 'very good agreement' = Kappa coefficient 0.8–1.0, 'perfect agreement' = Kappa coefficient 1.0.

Parameter	n	Relative observed agreement	Kappa coefficient	Agreement
Presence of perivitelline threads	56	0.96	0.84	Very Good agreement
Association with fragmentation	48	0.98	0.66	Good agreement
Cell stage	46	1	1	Perfect agreement

Please cite this article in press as: Ranya Derrick, Cristina Hickman, Oriol Oliana, Thomas Wilkinson, Danielle Gwinnett, Lucy Benham Whyte, Anna Carby, Stuart Lavery, Perivitelline threads associated with fragments in human cleavage stage embryos observed through time-lapse microscopy, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.08.026

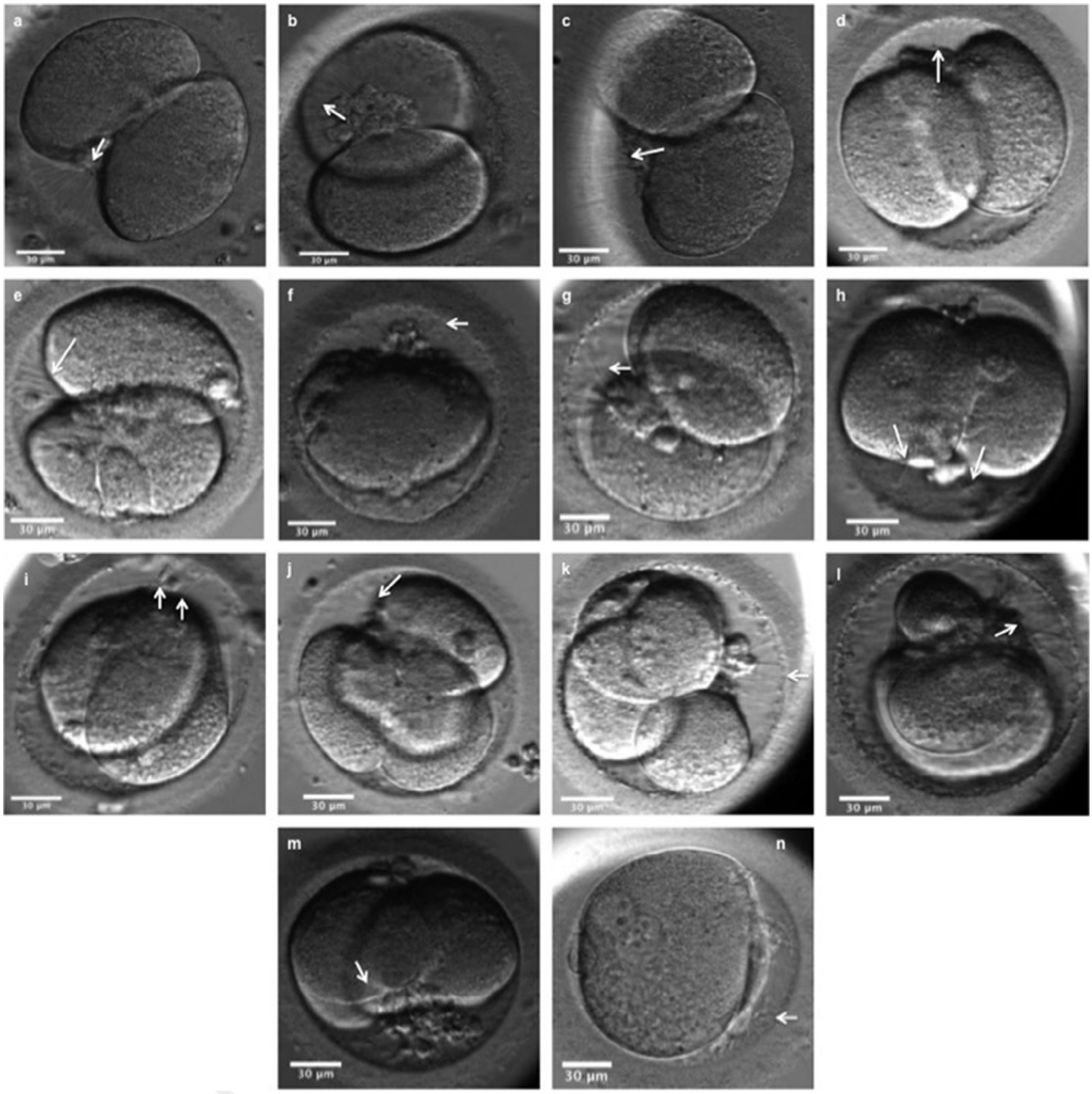

Figure 1 – A series of images illustrating either fans of perivitelline threads (PVT), or independent perivitelline threads within the embryo. Threads are indicated with a white arrow. The figures correspond to supplementary videos 1a–1n. Figure 1: (a) Perivitelline threads (PVT) can be observed in the cleavage plane of the first cleavage at 7'o'clock; (b) in the cleavage plane of the first cleavage at 10'o'clock; (c) in the cleavage plane of the first cleavage at 8'o'clock; (d) in the cleavage plane of the first cleavage at 12'o'clock; (e) in the cleavage plane of the first cleavage at 9'o'clock; (f) in the cleavage plane of the first cleavage at 12'o'clock, above both blastomeres; (g) in the cleavage plane of the first cleavage at 10'o'clock; (h) in the cleavage plane of the first cleavage at 7'o'clock; (i) in the cleavage plane of the first cleavage at 1'o'clock; (j) in the cleavage plane of the second cleavage at 11'o'clock; (k) in the cleavage plane of the third cleavage at 3'o'clock; (l) in the cleavage plane of the first cleavage at 2'o'clock; (m) in the cleavage plane of the first cleavage at 6'o'clock; (n) and at the one cell stage, starting at 6'o'clock to 1'o'clock.

Analysis of PVT

PVT are thin filaments that extend across the perivitelline space connecting the zona pellucida with the oolemma or blastomere membrane and can be categorized under the wider umbrella term of trans-zonal projections (TZP). They can occur either as several threads (greater than or equal to five threads) or a few threads (fewer than five threads (**Figure 1** and **Supplementary videos 1a–1n**). All seven focal planes were assessed. Contrast and saturation tools in the Embryoscope™ software (Vitrolife, Denmark) were used to enhance image quality to

Please cite this article in press as: Ranya Derrick, Cristina Hickman, Oriol Oliana, Thomas Wilkinson, Danielle Gwinnett, Lucy Benham Whyte, Anna Carby, Stuart Lavery, Perivitelline threads associated with fragments in human cleavage stage embryos observed through time-lapse microscopy, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.08.026

Table 3 – Proportion of embryos with and without perivitelline threads displaying each parameter analysed.

Parameter analysed	Proportion of embryos with PVT, % (n)	Proportion of embryos without PVT, % (n)	*P*-value
Incidence	77 (404/525)	23 (121/525)	
Fragmentation	96 (388/404)	67 (81/121)	$P < 0.001$
Implantation rate	25 (64/259)	28 (25/90)	NS
Euploid rate	40 (61/152)	49 (17/35)	NS

NS, non-significant.

improve PVT identification. The cell stage at which PVT were first seen was recorded, as well as whether the PVT seemed to aid in fragmentation (PVT associated with fragmentation was defined as fragments being located at the same site as the PVT and fragments moving in conjunction with the PVT). Fragments were defined as anuclear, cytoplasmic, membrane-bound structures, less than 40 µm (Johansson et al., 2003; Stensen et al., 2015). The degree of fragmentation was not analysed in this study.

Videos of the ICSI procedure were checked to assess whether PVT were already present at the time of injection, and general conclusions are described here without data.

Statistical analysis

Chi-square tests were carried out on all categorical variables to determine whether significant differences could be observed between treatments. Differences were considered significant if $P < 0.05$. Statistical analysis was carried out using Microsoft Excel™ for chi-square tests and an online calculator for the power analysis (Sample Size Calculator; http://clincalc.com/stats/samplesize.aspx). The 95% confidence intervals were calculated for each graph using the following formula: p + /– z* (sqrt)[(p(1–p)/n)] where p was the sample proportion, n was the sample size and z* was 1.96 for this CI (Rumsey, 2011).

Results

PVT were found in most (404/525 [77%]) embryos capable of blastulating. In most of these embryos (396/404 [98%]), PVT were observed at the two-cell stage. In most embryos with PVT (388/404 [95%]), PVT were observed to be directly involved in fragmentation.

Fragmentation occurred in 67% (81/121) of embryos in which PVT were not observed. Fragmentation occurred significantly more frequently in embryos in which PVT were observed compared with those that were not observed ($P < 0.001$). The implantation rate did not differ between embryos that had PVT (64/259 [25%]) and those that did not (25/90 [28%]). Similarly no significant difference were observed in euploidy rate between embryos that had PVT (61/152 [40%]) and those that did not (17/35 [49%]) (**Table 3**).

In ICSI videos, TZP could be observed at the oocyte stage, spanning from the corona cells, across the zona pellucida and attaching to the oolemma, examples of which are shown in the still images in **Figure 2**. Sub-zonal cellular debris were observed, clearly associated with these structures.

In this embryo cohort, 70% (366/525) were annotated for the fertilization method used. No significant variation was observed between the proportion of embryos displaying PVT between IVF (79/111 [71%]) and ICSI (190/255 [75%]) methods.

Discussion

This is the first published observation of PVT in human embryos, which is surprising given its high incidence in embryos: more than three-quarters of all embryos capable of blastulating had this feature. The increased use of time-lapse incubation for human embryos, the improvements in the optic contrast observed with the latest time-lapse incubators (Embryoscope Plus improvements over previous time-lapse incubators) and the visualization of all seven focal planes might have helped facilitate the identification of this structure within the cleavage stage embryo. It is expected that the true incidence may be even higher than that observed given the limitation that embryos could not be rotated within the time-lapse incubator and PVT may have occurred outside the view of the seven focal planes. Furthermore, identification of these structures was difficult owing to their transient nature and varying appearance.

This study was first presented at the Annual meeting for the British Fertility Societies (Fertility 2017, Edinburgh), and a second independent abstract reported on the same feature (Kellam et al., 2017). Kellam et al. (2017) agreed with our findings in observing PVT in most embryos, mostly at the two-cell stage and closely associated with fragmentation. The present study focused on embryos that were capable of blastulating, and was not designed to identify incidence of PVT in all embryos; Kellam et al., (2017), however, randomly selected a range of embryos of varying levels of viability and morphology,

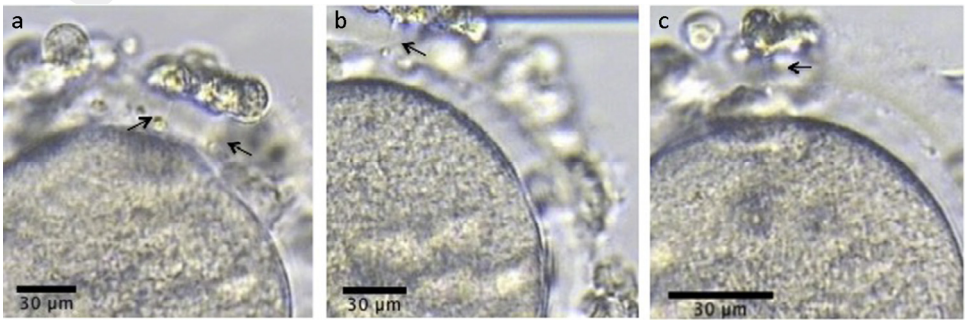

Figure 2 – A series of images from an intracytoplasmic sperm injection video showing trans-zonal projections spanning from the corona cells and across the zona pellucida, (indicated by black arrows).

Please cite this article in press as: Ranya Derrick, Cristina Hickman, Oriol Oliana, Thomas Wilkinson, Danielle Gwinnett, Lucy Benham Whyte, Anna Carby, Stuart Lavery, Perivitelline threads associated with fragments in human cleavage stage embryos observed through time-lapse microscopy, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.08.026

and, in agreement with the present, concluded that PVT occurred in most embryos and were closely associated with fragmentation. Similar to PVT, fragmentation is also observed in most embryos, and rarely occurs after genomic activation, which normally occurs around the four-cell stage in humans (Alikani et al., 2005). Some research also supports the fact that fragmentation most abundantly occurs at the first mitotic division (Fujimoto et al., 2011; Van Blerkom et al., 2001). The frequency, timing of appearance and the physical attachment between PVT and fragments highlights the association observed between these two features.

One possibility is that PVT could originate from the corona radiata. Corona radiata cells have projections that traverse the zona pellucida to communicate with the oolemma before ovulation. Corona radiata play an important role in providing the oocyte with the necessary resources, and organelles, for development and future viability (Gilula et al., 1978; Li and Albertini, 2013). At the time of the LH surge, these projections are withdrawn, thus removing inhibtory cyclic AMP signals and allowing for occyte maturation to recommence (Allworth and Albertini, 1993; Barrett and Albertini, 2010; Li and Albertini, 2013).

Although these studies suggest that these projections do not persist beyond this meiotic reactivation stage, coronal projections through the zona pellucida observed during ICSI (**Figure 2**) clearly demonstrate that remnants of these projections can persist and, therefore, PVT may represent persistent projections of corona radiata cells. This is an important observation, as these data suggest that perhaps not all TZPs are withdrawn at the resumption of meiosis, but that their persistence has no developmental consequence to implantation potential or ploidy. Furthermore, one could expect that PVT may be influenced by the fertilization method used; however, our results show no significant difference between ICSI and IVF procedures and the presence of PVT. It has been proposed that weaknesses in the oocyte membrane, the cytoskeleton, or both, can increase the risk of embryo fragmentation (Fujimoto et al., 2011). Therefore, an alternative possibility could be that PVT may be associated with fragmentation owing to their connection with the cellular membrane: as the zygote pulls away from the zona pellucida during the first cytokinesis of the first mitotic division, tight adherence between the PVT and the membrane may cause a strain during movement of the cells, causing fragments to form where there are already some weaknesses. This would be consistent with our findings that PVT were most often associated with fragmentation at the first cell division. A third possibility is that PVT association with fragmentation is incidental, and that fragmentation in the cleavage furrow is part of a normal process of the membrane dynamics of the first cell division, although this would not explain the significant association between fragmentation and PVT observed in this study.

Although increased fragmentation has been associated with reduced implantation potential (Holte et al., 2007; Montag et al., 2013) and an embryonic response to aneuploidy (Chavez et al., 2012), no significant relationship was observed between PVT and implantation potential or ploidy status, suggesting no significant relationship between PVT and embryo viability. Chavez et al. (2012) explored the idea that fragments can contain chromosomal material, and may even contain micronuclei. Whether this exclusion is a mechanism of aneuploid rescue is something that needs to be investigated in the future. As defined by Mattila and Lappalainen (2008), filopodia are thin, actin-rich plasma-membrane protrusions (Fierro-González et al., 2013; Mattila and Lappalainen, 2008). Studies have shown that transzonal processes are rich in microtubule and actin components (Li and Albertini, 2013), whereas filopodia have been found to also contain myosin, E-cadherin and catenins (Fierro-González et al., 2013). Although outside the scope of this study, further structural analysis of PVT could clarify whether PVT originate from outside the cells or from within, and would further clarify the function and role of PVT in early embryonic development.

In the embryos in which hreads were not observed, fragments were still present, suggesting that, despite the association between PVT and fragmentation, PVT are not essential for fragmentation to occur.

Salas-Vidal and Lomelí (2004) described filopodia which extend through the blastocoelic cavity to connect mural trophectoderm cells with cells of the inner cell mass. Hardarson et al. (2012) confirmed these findings in their work. These studies suggested that filopodia seemed to facilitate signal transmission and communication between these cell groups. Filopodia have also been found to aid in modifying blastomere shapes to allow for effective compaction; a key event in blastocyst formation (Fierro-González et al., 2013). These studies only describe intra-embryonic projections within mouse embryos, with no described association with fragmentation, although fragmentation in the mouse embryo is extremely rare. In contrast, PVT may originate from the corona radiata, are located in the perivitelline space, are observed early in development and seem to have a strong relationship with fragmentation. Therefore, although, previous literature has described thread-like structures within the embryo, these structures seem to have a different function, source, location and appear at a different stage of development compared with PVT.

Further work is under way to identify the degree and type of fragmentation associated with PVT, the number, length, longevity of PVT and their timing of appearance and positioning in relation to cell division. These were outside the scope of the present study.

In conclusion, PVT are a feature of embryo development that has not been previously reported. PVT are present in most human embryos, mainly found at the two-cell stage. Although they do not seem to influence implantation or ploidy, they have a strong association with fragmentation.

Acknowledgement

This study was conducted jointly by Imperial College London (Faculty of Medicine) and Boston Place Clinic (part of the Fertility Partnership). No further funding was required for this study.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.rbmo.2017.08.026.

ARTICLE INFO

Article history:
Received 30 January 2017
Received in revised form 18 August 2017
Accepted 23 August 2017
Declaration: The authors report no financial or commercial conflicts of interest.

Keywords:
corona radiata
fragmentation
IVF
morphokinetics
perivitelline threads
time-lapse

REFERENCES

Alikani, M., 2005. Epithelial cadherin distribution in abnormal human pre-implantation embryos. Hum. Reprod. 20, 3369–3375. doi:10.1093/humrep/dei242.

Alikani, M., Cohen, J., Tomkin, G., Garrisi, G.J., Mack, C., Scott, R.T., 1999. Human embryo fragmentation in vitro and its implications for pregnancy and implantation. Fertil. Steril. 71, 836–842.

Alikani, M., Schimmel, T., Willadsen, S.M., 2005. Cytoplasmic fragmentation in activated eggs occurs in the cytokinetic phase of the cell cycle, in lieu of normal cytokinesis, and in response to cytoskeletal disorder. Mol. Hum. Reprod. 11, 335–344. doi:10.1093/molehr/gah171.

Allworth, A.E., Albertini, D.F., 1993. Meiotic maturation in cultured bovine oocytes is accompanied by remodeling of the cumulus cell cytoskeleton. Dev. Biol. 158, 101–112. doi:10.1006/dbio.1993.1171.

Barrett, S.L., Albertini, D.F., 2010. Cumulus cell contact during oocyte maturation in mice regulates meiotic spindle positioning and enhances developmental competence. J. Assist. Reprod. Genet. 27, 29–39. doi:10.1007/s10815-009-9376-9.

Campbell, A., Fishel, S., Bowman, N., Duffy, S., Sedler, M., Fontes, C., Hickman, L., 2013. Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. Reprod. Biomed. Online 26, 477–485. doi:10.1016/j.rbmo.2013.02.006.

Chavez, S.L., Loewke, K.E., Han, J., Moussavi, F., Colls, P., Munne, S., Behr, B., Reijo Pera, R.A., 2012. Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. Nat. Commun. 3, 1251. doi:10.1038/ncomms2249.

Desai, N., Ploskonka, S., Goodman, L.R., Austin, C., Goldberg, J., Falcone, T., 2014. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. Reprod. Biol. Endocrinol. 12, 1–10. doi:10.1186/1477-7827-12-54.

Fierro-González, J.C., White, M.D., Silva, J.C., Plachta, N., 2013. Cadherin-dependent filopodia control preimplantation embryo compaction. Nat. Cell Biol. 15, doi:10.1038/ncb2875.

Fragouli, E., Alfarawati, S., Spath, K., Wells, D., 2014. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. Mol. Hum. Reprod. 20, 117–126. doi:10.1093/molehr/gat073.

Fujimoto, V.Y., Browne, R.W., Bloom, M.S., Sakkas, D., Alikani, M., 2011. Pathogenesis, developmental consequences, and clinical correlations of human embryo fragmentation. Fertil. Steril. 95, 1197–1204. doi:10.1016/j.fertnstert.2010.11.033.

Gilula, N.B., Epstein, M.L., Beers, W.H., 1978. Cell-to-cell communication and ovulation. A study of the cumulus-oocyte complex. J. Cell Biol. 78, 58–75.

Hardarson, T., Van Landuyt, L., Jones, G., 2012. The blastocyst. Hum. Reprod. 27 (Suppl. 1), i72–i91. doi:10.1093/humrep/des230.

Hardy, K., Stark, J., Winston, R.M.L., 2002. Maintenance of the inner cell mass in human blastocysts from fragmented embryos. Biol. Reprod. 68, 1165–1169. doi:10.1095/biolreprod.102.010090.

Holte, J., Berglund, L., Milton, K., Garello, C., Gennarelli, G., Revelli, A., Bergh, T., 2007. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum. Reprod. 22, 548–557. doi:10.1093/humrep/del403.

Johansson, M., Hardarson, T., Lundin, K., 2003. There is a cutoff limit in diameter between a blastomere and a small anucleate fragment. J. Assist. Reprod. Genet. 20, 309–313.

Kellam, L., Pastorelli, L., Campbell, A., 2017. Observation of commonly occuring zona-cytoplasmic threads (ZCTs) in early embryo development. Fertil. 2017 Poster 130.

Keltz, M.D., Skorupski, J.C., Bradley, K., Stein, D., 2006. Predictors of embryo fragmentation and outcome after fragment removal in in vitro fertilization. Fertil. Steril. 86, 321–324. doi:10.1016/j.fertnstert.2006.01.048.

Kovacic, B., Vlaisavljevic, V., Reljic, M., Cizek-Sajko, M., 2004. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. Reprod. Biomed. Online 8, 687–694.

Li, R., Albertini, D.F., 2013. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. Nat. Rev. Mol. Cell Biol. 14, 141–152. doi:10.1038/nrm3531.

Mattila, P.K., Lappalainen, P., 2008. Filopodia: molecular architecture and cellular functions. Nat. Rev. Mol. Cell Biol. 9, 446–454. doi:10.1038/nrm2406.

Montag, M., Toth, B., Strowitzki, T., 2013. New approaches to embryo selection. Reprod. Biomed. Online 27, 539–546. doi:10.1016/j.rbmo.2013.05.013.

Mumusoglu, S., Yarali, I., Bozdag, G., Ozdemir, P., Polat, M., Sokmensuer, L.K., Yarali, H., 2017. Time-lapse morphokinetic assessment has low to moderate ability to predict euploidy when patient– and ovarian stimulation–related factors are taken into account with the use of clustered data analysis. Fertil. Steril. 107, 413–421, e4. doi:10.1016/j.fertnstert.2016.11.005.

Prados, F.J., Debrock, S., Lemmen, J.G., Agerholm, I., 2012. The cleavage stage embryo. Hum. Reprod. 27, i50–i71. doi:10.1093/humrep/des224.

Rumsey, D.J., 2011. Statistics for dummies, 2nd ed. Wiley.

Salas-Vidal, E., Lomelí, H., 2004. Imaging filopodia dynamics in the mouse blastocyst. Dev. Biol. 265, 75–89. doi:10.1016/j.ydbio.2003.09.012.

Sample Size Calculator, n.d. [WWW Document]. http://clincalc.com/stats/samplesize.aspx. (Accessed 20 May 2016).

Sermon, K., Capalbo, A., Cohen, J., Coonen, E., De Rycke, M., De Vos, A., Delhanty, J., Fiorentino, F., Gleicher, N., Griesinger, G., Grifo, J., Handyside, A., Harper, J., Kokkali, G., Mastenbroek, S., Meldrum, D., Meseguer, M., Montag, M., Munné, S., Rienzi, L., Rubio, C., Scott, K., Scott, R., Simon, C., Swain, J., Treff, N., Ubaldi, F., Vassena, R., Vermeesch, J.R., Verpoest, W., Wells, D., Geraedts, J., 2016. The why, the how and the when of PGS 2.0: current practices and expert opinions of fertility specialists, molecular biologists, and embryologists. Mol. Hum. Reprod. 22, 845–857. doi:10.1093/molehr/gaw034.

Stensen, M.H., Tanbo, T.G., Storeng, R., Åbyholm, T., Fedorcsak, P., 2015. Fragmentation of human cleavage-stage embryos is related to the progression through meiotic and mitotic cell cycles. Fertil. Steril. 103, 374–381, e4. doi:10.1016/j.fertnstert.2014.10.031.

Stone, B.A., Greene, J., Vargyas, J.M., Ringler, G.E., Marrs, R.P., 2005. Embryo fragmentation as a determinant of blastocyst development in vitro and pregnancy outcomes following embryo transfer. Am. J. Obstet. Gynecol. 192, 2014–2019. doi:10.1016/j.ajog.2005.02.048.

Van Blerkom, J., Davis, P., Alexander, S., 2001. A microscopic and biochemical study of fragmentation phenotypes in stage-appropriate human embryos. Hum. Reprod. 16, 719–729.

Zhan, Q., Ye, Z., Clarke, R., Rosenwaks, Z., Zaninovic, N., 2016. Direct unequal cleavages: embryo developmental competence, genetic constitution and clinical outcome. PLoS ONE 11, e0166398. doi:10.1371/journal.pone.0166398.

Please cite this article in press as: Ranya Derrick, Cristina Hickman, Oriol Oliana, Thomas Wilkinson, Danielle Gwinnett, Lucy Benham Whyte, Anna Carby, Stuart Lavery, Perivitelline threads associated with fragments in human cleavage stage embryos observed through time-lapse microscopy, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.08.026